are cut in the same way and then placed on pedestals. When the whole active part of the seedling is under water growth ceases, but in moist air they do very well. Seeds kept some months in the very dry air of the laboratory refuse to germinate.—EDWIN BINGHAM COPELAND, *Stanford University, California.*

CONTRIBUTIONS TO THE BIOLOGY OF RHIZOBIA.

III. NOTES ON THE WINTER AND EARLY SPRING CONDITIONS OF RHIZOBIA AND ROOT TUBERCLES.

THE major observations here recorded were made during the winter and early spring of 1893 and 1894; incidental observations were also made during subsequent winter seasons. The object was to obtain more definite information regarding the permanency of leguminous root tubercles and the viability and natural resistance of rhizobia to low temperatures, more especially low temperatures with frequent changes to higher temperatures as in the winter months of the central states, Illinois in particular. Sudden changes of temperature, though not necessarily fatal to life, have a pernicious effect upon low organisms. The effects of temperature and other climatic conditions become gradually less with increase in depth of soil. The organisms found in the surface soil are most directly exposed to the pernicious climatic changes.

From the following table of mean monthly averages for the months of November, December, January, February, and March, 1902–3, with a list of coldest days of each month, it will be seen that the variation in temperature was considerable. Very cold weather (— 10° to — 15° F.) did not continue for more than a few days at a time.

Month	Min. ° F.	Max. ° F.	Mean ° F.	Coldest days ° F.
November	25.9	52.1	37.3	6, 10, 14, 16, 18, 18.
December	19.9	38.4	30.0	— 6, 1, 3, 5, 6, 6, 8, 9.
January	20.3	39.7	29.4	—21, —12, 12, 10, 12.
February	15.6	33.9	24.7	— 5, 2, 3, 3, 7, 8.
March.....	32.9	55.6	43.5	10, 11, 12, 16, 21.

The humidity during the months indicated was somewhat above the average for the state of Illinois. The ground was covered with snow during parts of the months of January, February and March. Soil covered by snow banks was not frozen at any time. The open unprotected soil was frozen to a depth of about eighteen inches during January, February and the early part of March.

The prevailing opinions held have been that leguminous root tubercles are destroyed at the close of the seasonal vegetative period and that the cytoplasmic and albuminoid contents of rhizobia are absorbed or assimilated by the host plant. Upon examining the roots of *Trifolium pratense* late in November it was found that the tubercles present were normal in appearance. Microscopic examination showed that rhizobia (*R. mutabile*) were present in smaller numbers than during the summer months. The highly refractive sporoids (fatty particles) were more plentifully present and more distinct. Since the tubercles were intact the question arises, what became of the missing rhizobia? It is highly probable that through lack of nutriment they became famished and finally died. Most organisms present reacted very feebly with the usual cytoplasmic stains, indicating a reduction in the cytoplasm. In such rhizobia the above mentioned sporoids were very distinctly visible and took the stain readily. Each organism showed from one to five such sporoids, more usually one in the neck portion and two or sometimes three or four in the body of the Indian club-shaped rhizobia, and quite generally occupying a position next to the cell-wall. They are not uniform in size and form. They stain a reddish brown with iodin tincture and are very clearly shown in an aqueous solution of corrosive sublimate. Infecting threads (Infectionsfäden) are present in apical areas and show no special modifications. In some tubercles they seemed to be wholly wanting. When present they are usually very distinct for several reasons, because of the lesser abundance of rhizobia and also because of the greater thickness of the cellulose wall. They are almost entirely empty, containing only a few small motile forms of the rhizobia. The walls of the filaments attain their maximum thickness late in the fall of the first season; the following season they do not increase in thickness, though they become refilled with motile rhizobia, finally rupturing the wall or escaping through breaks already existing. Thus they again refill the cells with mature, greatly modified, non-motile rhizobia. During the second year's growth of the tubercles, the filaments often disappear entirely. They may be destroyed or assimilated by the rhizobia or by the host plant. In some instances the filaments become separated from the cell-walls because of the tension due to the growing cell. The separation may take place in any part of the threads, but more commonly where they unite with the cell-wall. During the second season the partially emptied infected area of tubercles again becomes tensely filled with mature rhizobia, through the multiplication of organisms

found in the infecting threads which are found in the apical areas of tubercles and in the cells just within the phellogenic layers. Additions to the growth of the tubercles are also made at these points. The starch, which was deposited just outside of these meristematic areas in the fall of the year, is now again assimilated by the host plant.

It is evident that perhaps about one-half, or somewhat less, of the rhizobia existing within the infected areas of tubercles are killed during the unfavorable winter conditions. Freezing alone does not kill them; it is rather a combination of conditions, the lack of food supply perhaps being the most important. The tubercles and soil examined were taken from near the surface of the frozen ground when the temperature was from -10° to -20°F. Cultures were made from the tubercles as well as from the soil by the usual plate isolation methods. The growths showed the presence of rhizobia and other soil bacteria. Streak and stab cultures were also made from the infected area of tubercles. A careful examination of growths and culture media at the point of inoculation showed a number of impoverished tubercle organisms which had evidently lost the power of dividing. These stained very feebly and the cell-wall was partially destroyed, having a roughened perforated appearance. Soil cultures showed the presence of rhizobia, besides numerous soil bacteria.

Examining tubercles which were more deeply situated, about one foot below the surface of the soil, showed that the destruction of rhizobia had been less and the number of dead but not destroyed rhizobia was also less, which would seem to indicate that cold was also a factor to be considered in the killing of rhizobia. It seems probable that rhizobia of tubercles below the freezing depth develop and multiply to some extent, though the tubercles do not increase in size, as is indicated by the tensely filled glistening appearance of such tubercles. A careful examination of a number of such tense, brittle tubercles showed that they contained numerous rhizobia imbedded in a large amount of a mucilaginous substance. The cells of the infected area were loosely united and almost spherical in form. The contents of these tubercles require further study.

The observations were made chiefly upon tubercles of *Melilotus alba*, *Trifolium pratense*, and *T. repens*, and the conclusions with reference to these plants are that root tubercles are mostly biennial, the tubercles attaining their full growth during the first year and gradually dying and decaying toward the close of the second year.[2] With the

[2] New tubercles are, of course, added each season along with the development of new rootlets.

death and decay of the tubercles most of the contained rhizobia also die, but some escape into the soil and serve to infect other roots of the same host species. No comparisons were made between summer and winter soil to determine the comparative number of rhizobia present. It is, however, highly probable that the conditions are much as with the rhizobia of summer and winter tubercles. The tubercles of annuals, like the bean, pea, Spanish pea, etc., die and decay at the close of the vegetative period and many of the contained rhizobia escape into the soil. Many are no doubt killed and assimilated by the host plant shortly before the close of the vegetative period; according to some authorities during the seed- and fruit-forming period of the host plant.

The following are the conclusions based upon the observations recorded:

1. A considerable number of rhizobia of biennial and perennial plants forming root tubercles are killed during the winter months.

2. Root tubercles of perennial herbaceous legumes attain their full growth during the early part of the first season.

3. Most root tubercles of perennial herbaceous legumes die and decay at the close of the second season, returning only a part of the contained rhizobia to the soil. Many of the rhizobia are assimilated by the host plant during the period of fruit development.—ALBERT SCHNEIDER, *California College of Pharmacy, San Francisco.*